OSTEOGENESIS OF DENTAL STEM CELLS

NOVA SOUTHEASTERN UNIVERSITY COLLEGE OF DENTAL MEDICINE

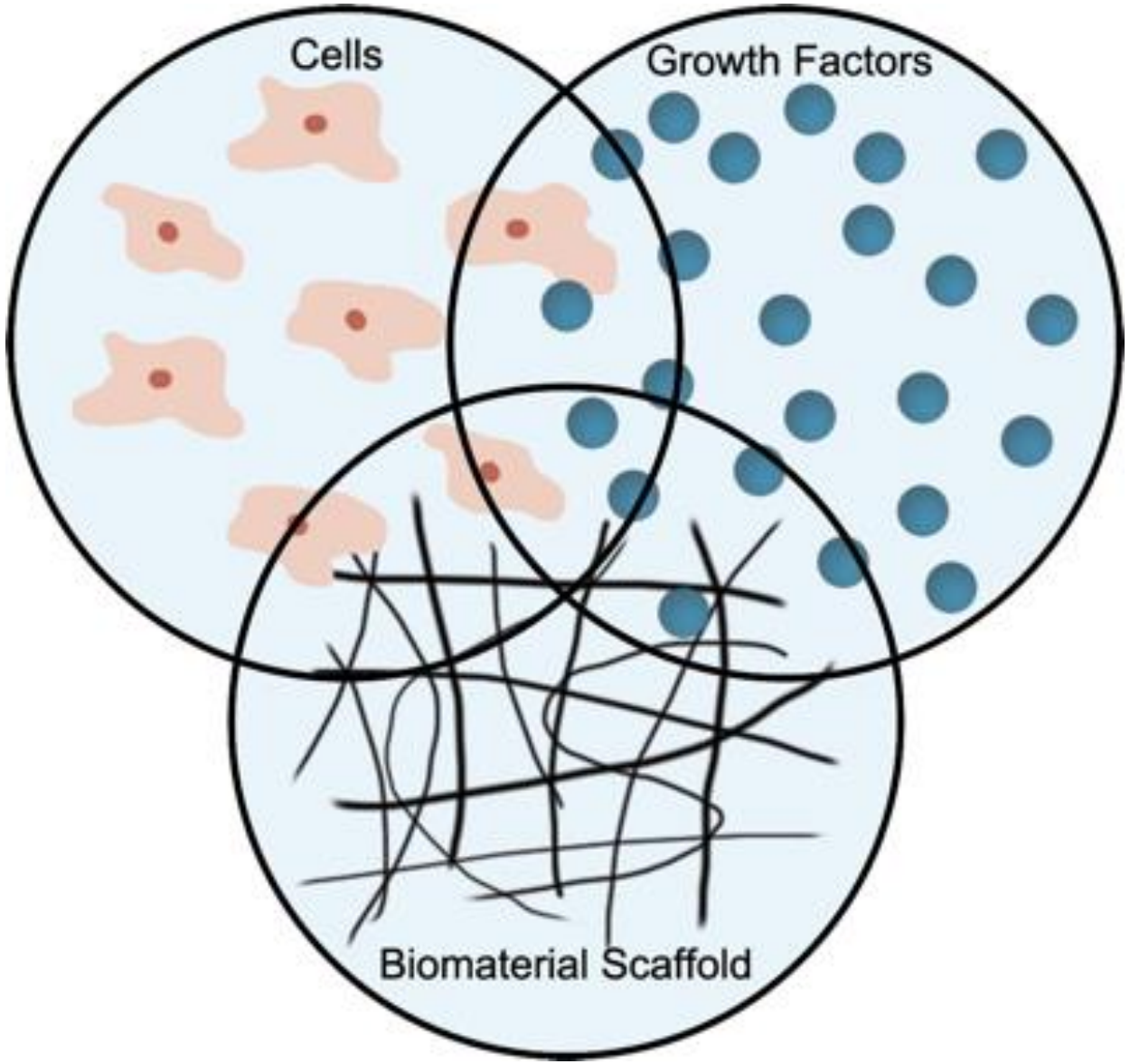
BIOL4990-INDEPENDENT STUDY

DR. UMADEVI KANDALAM

JERRY ENNOLIKARA

RESEARCH LABORATORY OVERVIEW

- This research lab is dedicated to the study of bioengineering bone tissue, an application of biology research within medicine known as "regenerative medicine", through a variety of in-vivo and in-vitro projects.
- The primary focus of our lab is to replace a lifetime of bone graft surgeries associated with cleft palate, a congenital disorder where the palatal process of the maxilla fails to fuse during development, with a noninvasive injectable treatment
- The lab upholds the central motif of tissue engineering of "cells, scaffolds, and growth factors" with our research utilizing periodontal ligamental stem cells/human gingival mesenchymal stem cells; 3D hydrogel scaffolds such as Puramatrix™ and Viagraft™ or bone powder; and osteogenic growth factors such as BMP-2 and more recently, FGF and VEGF in our newer studies
- The research is focused in a dental maxillofacial model yet also has many implications for medicine within the orthopedic field.


Cells
Growth Factors
Biomaterial Scaffold

STUDENT RESEARCHER EXPERIENCES

- The lab is located primarily within the College of Dental Medicine, yet students ranging from the pre-dental to the pre-medical track work in the lab.
- Student researchers have the opportunity to work and learn in many cross-curricular laboratory processes/techniques and connect the skills learned from the lab sciences to the real dental and medical field, such as:
  - Genetics: PCR, Real Time PCR
  - Biochemistry: Western blotting, Enzymatic assaying
  - Microbiology: Cell culturing, Cell assaying, Cell staining
  - Histology: Microscopy, Slide preparation, Tissue analysis
- Most students researcher participate in research-for-credit by setting up and registering for a BIOL4990 Independent Study course section (1-3 credits) with the Biology department
- Each credit is valued to three hours of lab work for every week, or else otherwise as coordinated with Dr. Kandalam (or whoever your research professor is; this process is general for most research-for-credit courses)

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of Dental Stem
fibrous Hydrogel

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Dr. Umadevi Kandalam
ma Southeastern University

Cleft Palate

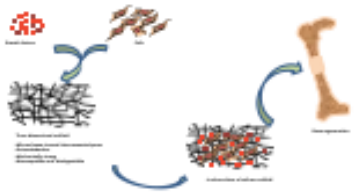
- Most common birth defect
- One in 700 births in the US
- Incomplete fusion of two palatal shelves
- Only current treatment: multiple surgeries within first two years, multiple follow up surgeries throughout life

2

TISSUE ENGINEERING

3

Tissue Engineering



4

Long Term Goal

Repair and regenerate bone in the cleft palate region.

Study Objective

Develop injectable scaffold systems with stem cells to regenerate bone tissue.

5

Cells: Periodontal Ligament Stem Cells

- Mesenchymal stem cells (MSCs) associated with periodontal regeneration
- Basic cell type used throughout project

6

Scaffold: Hydroge

7

Methodology

1. **PDLSCs were isolated and tested for surface markers.**
2. Cell proliferation was measured by WST assay over intervals of time (1, 2, 3 days)
3. Viability was measured with Live/Dead cell assays.
4. Gene expression of alkaline phosphatase (ALP) was measured by qPCR
5. Differentiation was observed by measuring ALP enzyme activity.

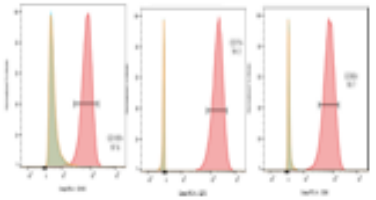
9

Detection of Surface Markers: Flow Cytometry

- International Society for Cellular Therapy (ISCT) sets standard for a cell population to be MSCs
- Surface markers CD105+, CD73+, and CD90+ must be identified from flow cytometry

10

Results



11

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12

Cell Proliferation: Encapsulation in Hydrogel

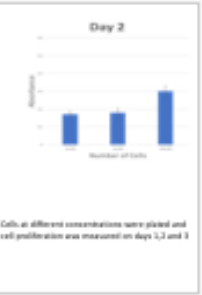
- 10,000x, 30,000x, and 800,000x PDLSCs were added to Puramatrix™ solution
- Culture medium added for peptide units to assemble into 3D matrix
- 3-D encapsulated cells were grown and used for WST assay

13

WST Assay

- Colorimetric assay performed after 1, 2, and 3 days
- Oxidoreductase enzymes reduce WST-1, a tetrazolium salt
- Depth of orange color from metabolite indicates amount of proliferation

14



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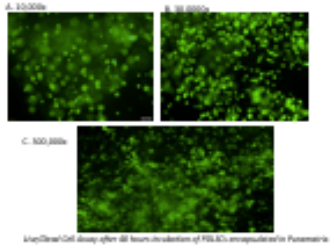
16

Cell Viability: Live/Dead Cell Assay

- Images captured highlight cell viability/death
- Light sensitive assay utilizing calcein-AM and ethidium homodimer (EthD) to dye live and dead cells, respectively.
- Composite images show only live cells

17

Results



18

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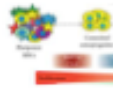
19

Differentiation: Complete vs. Osteogenic Medium

- Two million cells per gel were encapsulated.
- Cells grown in complete medium (CM) and osteogenic medium (CM with Ascorbic acid, β-Glycerophosphate, Dexamethasone)
- Samples were tested for differentiation markers.

20

Osteogenic Dif

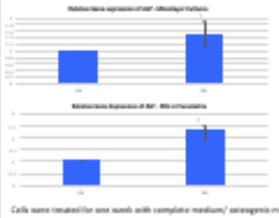


- Mesenchymal stem c
differentiate into mat
This leads to bone for
- Differentiation begins

21

- Real-time PCR determines the level of gene expression.

Results



Cells were treated for one week with complete medium/ osteogenic medium

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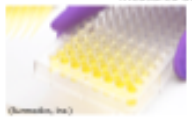
Osteogenic Differentiation: ALP Enzyme Activity

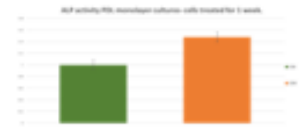
- Alkaline phosphatase (ALP) enzyme activity indicates differentiation
- pNPP assay indicates amount of ALP enzyme activity
- Cells treated for 1 week, ALP activity was measured on day 7



P-Nitrophenylphospate (pNPP) Assay

- Hydrolysis of p-Nitrophenylphosphate (pNPP) by alkaline phosphatase creates **P-NITROPHENOL**
- Cells grown in complete medium (CM) and osteogenic medium (OM) treated with pNPP
- Absorbance results indicate ALP enzyme activity

Results



Cells were treated with complete medium/osteogenic medium for 1 week and ALP activity was measured using pNPP assay

Conclusio

- Puramatrix™ s
growth and di
- This tissue eng
the potential a
regeneration

HOW TO GET INVOLVED

- The lab is open to any undergraduate students who are passionate about the lab sciences.
- To get involved, please email Dr. Umadevi Kandalam at umadevi@nova.edu with your resume and a letter of interest. From there, she or someone else will get back to you to potentially set up a date to interview with Dr. Kandalam herself and see the lab!
- If you have any other questions, please email me (Jerry Ennolikara) at je788@mynsu.nova.edu
- Additional contacts:
  - Nicole DeLorenzo: ndelorenzo@mynsu.nova.edu (Lab Manager)
  - Shreya Patel: sp1743@mynsu.nova.edu (Student Researcher)